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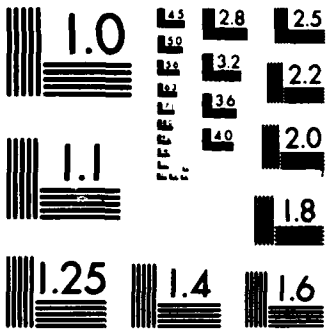

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19. ABSTRACT (Continue on reverse if necessary and identify by block number) This project is evaluating the effect of housing 1 or 5 mice in identical sized cages on immunologic function. We have found that the C3H/HeJ male mouse, when placed from group housing to 1 animal housed per cage, has an enhanced T cell related immune function in comparison to mice housed 5 per cage. Female C3H mice do not show this effect. The effect is present approximately 10 days after being placed into a single cage and lasts approximately 3-4 weeks. The altered immune reactivity is not related to corticosterone levels. The C3H.SW/SN mouse also shows a similar immunologic change. The SWSN mouse shares either the H_2-D or H_2-K loci antigens with the C3H/HeJ mice. Animals housed 1 per cage are significantly more resistant to infection with Candida albicans than animals housed 5 per cage. The time course of the development of enhanced resistance is similar to other immunologic parameters measured, appearing after approximately 10 days and disappearing at approximately 3-4 weeks. Thus, housing can influence immunologic parameters in some strains of mice and the immunologic alteration is most likely due to the change in housing conditions rather than the actual housing conditions present.

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ANNUAL REPORT

The Effective Housing (Number of Mice/Cage) on Immunologic Competency

ONR Contract No. N0O014-86-K-0500

1. INTRODUCTION

The immune system is susceptible to functional alteration by hormonal factors produced directly or indirectly by the central nervous system. One factor which may influence the immune response by central nervous system mediation in animals, is the number of animals housed per cage. For example, the response to transplanted tumors, the reaction to infectious agents, or the immune response to exogenous antigen, are each influenced by the number of animals housed per cage. The housing effect has been observed in mice, rats and guinea pigs and different strains of in-bred mice. We initially observed that C3H/HEJ male mice housed 1 per cage have enhanced T cell responsiveness when compared to mice of the same strain housed 5 per cage. Interleukin-2 release, Concanavalin A responsiveness, and the antibody response to a T dependent antigen were all significantly increased in the animals housed 1 per cage. B cell responsiveness did not differ based on housing.

The effect of housing on immune competency is not well understood. Our search of the available literature did not reveal any comprehensive immunologic studies of the mechanisms responsible for the observed phenomena. The goal of our research is to understand why the immune response differs under differential housing conditions. This would provide a basis for the subsequent systematic exploration of the psychological and behavioral factors that may be influencing the differential housing effects observed.

2. RESEARCH OBJECTIVES FOR YEAR 1

There were 4 goals for year 1:

1. Determine the effect of housing mice 1 or 5 per cage on 5 strains of mice.

2. Determine if the kinetic response to antigen is altered by the differential housing conditions.

3. Determine when the effect of individual housing influences immune function and how long it persists after animals are placed into individual cages.

4. Determine the effect of differential housing on the anamnestic immune reaction.

3. PROGRESS REPORT (JULY 15, 1986 - JULY 14, 1987)

We have completed goals 1, 2 and 3 as listed above, are having difficulty with determining the effect on the anamnestic response, and have added additional studies regarding the effect of housing on the response to infectious disease.

1. Determine the effect of housing mice 1 or 5 per cage on 5 strains of mice - Five strains of mice housed individually or in groups of 5 were immunized with sheep erythrocytes to determine if the major histocompatibility complex was associated with altered immune reactivity based on housing. In addition, corticosterone levels were measured in each of the animals. The data are shown in the following Table.

TABLE 1

NUMBER OF SPLEEN LYMPHOCYTES PRODUCING ANTIBODY TO SHEEP ERYTHROCYTES AND CORTICOSTERONE LEVELS (2 CAGES OF 5/CAGE AND 10 OF 1/CAGE)

Strain	H2D or H2K Identical to C3H/HeJ	PFC/10^6 Lymphocytes 5/Cage	PFC/10^6 Lymphocytes 1/Cage	Corticosterone (ug/dl) 5/Cage	Corticosterone (ug/dl) 1/Cage
B10.Br	Both	368 ± 118*	555 ± 105	18.6 ± 6.4*	22.3 ± 6.1
C_3H. SW/SN	Neither	588 ± 153	932 ± 74++	16.0 ± 4.5	16.6 ± 2.9
B10. A/Sg SN	Same H_2^K	773 ± 193	755 ± 91	15.0 ± 1.2	8.9 ± 0.9++
C_3H-H-2^02/SFSN	Same H_2^D	721 ± 171	758 ± 206	18.0 ± 2.3	14.0 ± 1.8
C3H/HeJ	-	790 ± 114	1450 ± 270++	15.2 ± 1.3	15.8 ± 1.7

* Mean ± S.D.

++ $p<0.01$

Individually housed mice from 2 strains which shared neither the H_2D or H_2K loci produce more antibody forming spleen lymphocytes to sheep erythrocytes than group housed mice. corticosterone levels were not related to the level of the immune response. Thus, genetic factors related to the MHC do not influence alteration of the immune response which occurs with differential housing conditions.

2. Determine if the kinetic response to antigen is altered by the differential housing conditions - Animals were immunized and the number of spleen lymphocytes producing antibody to sheep erythrocytes determined 2, 3, 4, 5, 6, 7, 8 and 10

odes

or

days after immunization. The plaque forming cell response to sheep erythrocytes peaked at 4 days in animals housed either 1 or 5 per cage. Thus, the differential housing conditions do not alter the kinetics of the immune response to sheep erythrocytes. The responsiveness to Concanavalin A will be described below.

3. Determine when the effect of individual housing influences immune function and how long it persists after animals are placed into individual cages - A study was performed to determine how soon after animals were placed 1 in 5 per cage the differential effect on the immune system occurred, and, how long it persisted. The data shown in Tables 2 and 3 below.

   As can be seen, the effect is transient, developing approximately 8-10 days after the animals are separated, and persists for approximately 3-4 weeks. The Concanavalin A differential effect seems to persist slightly longer than the plaque forming cell response to sheep erythrocytes. This indicates that the effect of differential housing is due to change in housing conditions, rather than the actual type of housing environment that the animal is maintained in. It is thus anticipated, that an alteration of immunologic function, which is induced by environmental change, would be transient until the animal had adapted to the new environment.

4. Determine the effect of differential housing on the anamnestic immune reaction - We have restricted our assay of plaque forming cells to sheep erythrocytes to lymphocytes producing IgM antibody. We have not been able to identify a dose of sheep erythrocytes and time after primary immunization that will provide adequate numbers of IgM forming cells during the secondary response to complete this phase of this study.

STUDIES PERFORMED DURING YEAR 1, WHICH HAD BEEN PROPOSED TO BE DONE IN YEAR 2 - We initiated studies on the effect of sex hormones on the housing effect. When male animals are castrated no difference is detected in the plaque forming cell response to sheep erythrocytes in animals housed 5 or 1 per cage. Similarly, the Con A response is the same. The immune response of the animals housed 5 per cage increases in castrated male mice housed 5 per cage so that it approximates the immunologic response of animals housed 1 per cage. Removing the ovaries from female mice did not produce a difference in the immunologic response of animals which were differentially housed.

ADDITIONAL STUDIES PERFORMED BUT NOT IN ORIGINAL APPLICATION - An assay was established in which mice were infected with C.

TABLE 2

LYMPHOCYTE PROLIFERATIVE RESPONSE TO CONCANAVALIN-A IN MALE C3H/HeJ MICE HOUSED 1 OR 5 PER CAGE AND SACRIFICED ON DIFFERENT DAYS

DAYS HOUSED	% INCREASE IN CPM IN MICE HOUSED 1/CAGE
5	0
8	20
12	TECHNICAL ERROR
14	37
25	15
32	10

The data are expressed as a percentage rather than in absolute numbers as the sensitivity of the assay may have changed on different days.

TABLE 3

PLAQUE FORMING CELL RESPONSE TO SHEEP ERYTHROCYTES IN MALE C3H/HeJ MICE HOUSED 1 OR 5 PER CAGE AND SACRIFICED ON DIFFERENT DAYS

DAYS HOUSED	% INCREASE IN PLAQUE FORMING CELLS IN MICE HOUSED 1/CAGE
5	0
8	10
12	99
14	79
25	11
32	0

The data are expressed as a percentage rather than in absolute numbers as the sensitivity of the assay may have changed on different days.

albicans and the number of organisms required to infect 50% of the animals determined. The C3H/HEJ male mouse was found to be significantly more resistant to C. albicans infection when housed 1 per cage, in comparison to 5 per cage. Female C3H/HEJ mice were equally resistant to infection. Thus, the results of the infection studies parallel those of the Concanavalin A and immune response to sheep erythrocytes. A second strain of mouse, the CD-1 mouse, showed a similar differential effect on resistance to C. albicans in the male animals. If the animals are left differentially housed for two weeks, before being infected, the effect of housing on resistance to infection is no longer present. Thus, we have found that the immune response to an exogenous antigen, non-specific mitogen reactivity and resistance to an infectious agent, are similarly altered by housing some strains of animals either 1 or 5 per cage.

Studies have been initiated in rats to determine whether all compartments (thymus, spleen, peripheral blood lymphocytes, lymph nodes) respond in an identical manner to an exogenous stressor. In this case the exogenous stressor used was electric shock. Our preliminary studies have shown that not all of the compartments are equally altered in their immunologic reactivity following exposure to a stressor. The significance of this finding to the overall immunologic competency of an intact animal are under investigation.

3. PLANS FOR NEXT YEAR

C3H/HEJ male mice which have either been adrenalectomized, hypothosectomized, or treated with naltrexone will be studied. In this manner, we will be able to determine whether opioid substances, factors produced by the adrenal gland other than corticosterone, or substances produced by the pituitary, are associated with the altered immune reactivity when animals are differentially housed. In addition, we will be pursuing the reasons why there is a difference between male and female animals in regard to the differential housing effect. We believe, that our data to date have indicated that with the proper genetic background, changes in environment can influence immune function including resistance to infectious disease. However, once adaptation occurs to the changed environment, the immune system resumes its normal function. An additional stressor, placed upon an animal in an altered environment, or changing environments, may produce continued alteration of the immune system. However, our current investigations have not explored that possibility.

PUBLICATIONS:

Rabin BS, Lyte M, Hamill E. The influence of mouse strain and housing on the immune response, J. of Neuroimmunol., In Press.

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